REMARKS

The Office Action dated March 26, 2003 presents the examination of claims 1-14. Claims 1 are 9 are amended. The specification is amended to correct typographical errors. No new matter is inserted into the application.

Rejection under Obviousness-type Double Patenting

The Examiner rejects claims 1-14 under the judicially created obviousness-type double patenting rejection over claims 1-21 of U.S. Patent 6,372,960. Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

In order to overcome this rejection, Applicants file herewith a Terminal Disclaimer in compliance with 37 C.F.R. § 1.321(c), disclaiming the terminal portion of any patent that may issue from the present application. The Terminal Disclaimer is signed by a registered attorney of record in the present application in compliance with 37 C.F.R. § 3.73(b).

Applicants respectfully submit that the filing of the Terminal Disclaimer overcomes the obviousness-type double patenting rejection. Withdrawal thereof is therefore respectfully submitted.

Objection to the Claims

The Examiner objects to claim 9 for a typographical error. Claim 9 is amended in accordance with the Examiner's suggestions. Thus, the instant objection is overcome.

Rejection under 35 U.S.C. § 103

The Examiner rejects claims 1, 2 and 7-14 as obvious over EP 412,911 (Plant Genetic Systems) in view of Koziel et al. (1993) and Hartley et al. (1993). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

EP 412,911 teaches the native barstar gene and its use to restore fertility in plants comprising barnase. SEQ ID No. 1 (native barstar) of EP 412,911 has an AT content of about 51%, as mentioned in the present invention at least on page 21 (Example 2). As the Examiner points out, EP 412,911 does not suggest to modify the AT content of the native barstar gene to obtain a DNA sequence having less than 40% AT. Neither does EP 412,911 provide motivation to modify the AT content of the native barstar gene to contain less than 40% AT. In fact, the native barstar gene with an AT content of about 51% was found to be suitable for successfully restoring fertility in plants comprising barnase (see EP 412,911 and US 6,506,963). US 6,506,963 describes the commercially used barnase/barstar system, wherein the native barstar gene is used which has an AT content of about 51%.

Koziel et al. teaches corn transformation with a *Bacillus thuringiensis* gene which has been modified to contain 35% AT, while the native Bt coding region has an AT content of 62%. The motivation to modify (i.e., reduce) the AT content of the native Bt gene was that the native *Bacillus thuringiensis* gene (which had an AT content greater than 60%) failed to produce detectable protein levels in transgenic maize plants (p. 194, col. 2, paragraph 3).

The GC content of coding regions of plant genes was known to be about 50% (Fischhoff et al. US patent 5,500,365, column 4, paragraph 3). Further, plant genes and most bacterial genes, which have successfully been expressed in plants, have an AT content of 45-55% (Fischhoff et al. col. 9, paragraph 3). Non-plant genes, such as *Bacillus thuringiensis* genes and *Streptomyces* genes have an AT content which deviates significantly from the AT content found in plant genes. For example, *Bacillus thuringiensis* genes are very rich in AT (~62%) while *Streptomyces* genes have a very low AT content (~30-35% AT)(Fischhoff et al., col. 9, p. 3). For such genes, a clear motivation existed to modify the AT content in order to match the AT content of native plant genes, i.e. about 50%.

More importantly, Strauch et al. (US patent 5,276,268) describe the generation of a synthetic PAT gene with about 50% AT for expression in plants, in contrast to the native PAT gene from *Streptomyces* which has about 30% AT (US 5,276,268 p. 4, line 3-

6). This patent therefore teaches away from modification beneath 40% AT, as a gene with 30% AT was modified to have about 50% AT content. Hence, the prior art in general taught to adapt the G+C (or A+T) content to about 50%.

Thus, the motivation to modify the AT content in order to express a gene in plants, is always found in an "unsuitable" AT content (significantly above or below 50% AT) of the native gene. Applicants therefore respectfully submit that there was no motivation for a skilled artisan to modify the AT content of a gene which already had a "suitable" AT content of about 50%. There was, therefore, no motivation to modify the AT content of the native barstar gene (which already had an AT content of 51%) to contain less than 40% AT. Such a modification would in fact be expected to make the barstar gene less suitable for expression in plants, because an AT content of 50% was already viewed as suitable (as illustrated by US 5,500,365 and US 5,276,268). By analogy, there was no motivation to modify an *E. coli* gene with an AT content of 50% AT, as indicated by Fischhoff et al. (col. 9, p. 4, last sentence).

Further, Koziel et al. fails to suggest altering the AT content of any genes other than the *Bacillus thuringiensis* genes (with high AT content), and is silent with respect to altering the barstar gene. In addition, the alteration of the coding sequence as taught in Koziel et al. is undertaken strictly to enhance gene expression. Koziel et al. does not disclose or

suggest to modify the AT content if an adequate gene expression is already achieved with the wild-type sequence, and when the AT content is not high.

The Examiner further cites Hartley et al. (1993). Hartley et al. teaches that the barstar protein can be modified by substituting a variety of differentially-charged amino acids at a variety of residues without affecting protein function. Although Hartley et al. teaches barstar (single or double) mutants which are functional *in vitro* and in bacteria, Hartley et al. does not teach or suggest modifications to the barstar gene which improve its activity *in planta*. In particular, Hartley et al. fails to disclose or suggest modifications that result in a higher level of barstar protein and improved "restore capacity" when expressed in plant cells (p. 10, line 25 - p. 11, line 19). There is no disclosure in Hartley et al. to decrease the AT content of barstar resulting in increased capacity of the altered barstar to inhibit barnase in a plant cell. Hence, a skilled artisan would not be led to conclude that decreasing the AT content would be favorable.

Additionally, Applicants respectfully submit that none of the cited references disclose or suggest that the modifications of the barstar DNA disclosed in the present application, upon expression in a plant cell, improve the inhibition of barnase in a plant cell. In this regard, the Examiner is referred to at least Example 5 on pages 27 and 28 of the instant specification

in which improved inhibition of barnase in plant cells is described.

A proper obviousness rejection requires, *inter alia*, that the prior art: 1) provides a motivation for making the claimed invention, and 2) provides a reasonable expectation of successfully practicing the claimed invention. Both the motivation and the reasonable expectation of success must be founded in the prior art, not in Applicant's disclosure. In re Vaeck, 20 USPQ2d 1438 (Fed. Cir. 1991).

Applicants respectfully submit that the instant rejection does not satisfy the requirement of providing actual evidence of a motivation or suggestion in the prior art to modify the AT content of the native barstar gene to less than 40% AT, because none of the cited references, either alone or in combination, provide such a motivation. It would therefore not have been obvious for a skilled person to modify the native barstar gene as claimed. In fact, US patent 5,276,268 actually teaches away from decreasing the AT content of genes significantly below 50% for expression in plants. This is evidenced by the fact that a gene disclosed therein having a 30% AT content was modified to have an increased AT content of about 50% for expression in plants.

In summary, there was no motivation to improve expression of barstar in the prior art, since fertility restoration was not reported to be poor due to poor expression of barstar in plants, and barstar native DNA has an about 50% AT content, falling right

in the range that the prior art considers to be the optimal AT-content. In addition, the modification as made in the present application which resulted in a sequence of less than 40% AT content, unexpectedly improved the expression of the barstar protein in plants and also improved restoration of fertility. The Examiner's attention is directed to the conclusions in Examples 4, 5 and 6 for the positive results obtained in three different plant species.

Conclusion

Applicants respectfully submit that the above amendments and/or remarks fully address and overcome the rejections and objections of record. The instant claims are now in condition for allowance. Early and favorable action by the Examiner is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. 45,702) at the telephone number of the undersigned below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional

fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachments: Version with Markings to Show Changes Made
Terminal Disclaimer

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning on page 3, line 23, has been amended as follows:

Some of these mutants were shown to essentially retain the biological activity of the barnase and barstar as produced by Bacillus amyloliquefaciens [amyloliguefaciens]. However, at least two mutant barstars have been described that have no detectable barstar activity (Hartley, 1993, Biochemistry 32:5978-5984; Guillet et al, 1993, Current Biology 1:165-177).

The paragraph beginning on page 4, line 27, has been amended as follows:

The present invention further provides uses of the improved barstar DNAs and [improved] improved barstar proteins to neutralize barnases in plant cells, particularly with regard to restoration of male fertility to male-sterile lines.

The paragraph beginning on page 9, line 24, has been amended as follows:

A male sterile plant as used herein, contains a foreign "male-sterility locus" which contains the male-sterility gene S which when expressed in cells of the plant [make] makes the plant

male-sterile without otherwise substantially affecting the growth and development of the plant.

The paragraph beginning on page 10, line 12, has been amended as follows:

Sterility promoters that can be used in the male-sterility genes in the first parent line of this invention have been described before (EP 0,344,029 and EP 0,412,911). The sterility promoter can be any promoter but it should at least be active in stamen cells, particularly tapetum cells. Particularly useful sterility promoters are promoters that are selectively active in stamen cells, such as the tapetum-specific promoters of the TA29 gene of Nicotiana tabacum (EP 0,344,029) which can be used in tobacco, oilseed rape, lettuce, [cichory] chicory, corn, rice, wheat and other plant species; the PT72, the PT42 and PE1 promoters from rice which can be used in rice, corn, wheat, and other plant species (WO 92/13956); the PCA55 promoter from corn which can be used in corn, rice, wheat and other plant species (WO 92/13957); and the A9 promoter of a tapetum-specific gene of Arabidopsis thaliana(Wyatt et al., 1992, Plant Mol. Biol. 19:611-922).

The paragraph beginning on page 25, line 2, has been amended as follows:

Seven transgenic male fertile restorer rice plants of cultivar Chiyonishiki were obtained essentially as described in WO 92/13956 using plasmid pTS173 which contains the following chimeric genes: P35S-bar-3'g7 and PE1-wild-type barstar-3'nos. pTS173 is derived from pJVR3-E1 (WO 92/13956) by replacing the 35S promoter and the 3' untranslated end of the chimeric bar gene of pJVR3-E1 by the 35S promoter and the 3' untranslated end of the chimeric bar gene of pTTS24 as follows. From the T-DNA insert of plasmid pTTS24 (SEQ ID No. 7) a DNA fragment containing the 3' end of T-DNA gene 7 and part of the bar gene is amplified by PCR using the oligonucleotide primers CASOLX1 (SEQ ID No 8), which overlaps the KpnI site in the bar gene, and CASOLX2 (SEQ ID No 9). The PCR product is cleaved with AatII and KpnI, and ligated to the large fragment of plasmid pJVR3-E1 cleaved with AatII and KpnI. From the obtained plasmid, the smaller NcoI+NotI [fragment,] (containing P35S) is replaced by the corresponding NcoI-NotI fragment from pTTS24 (positions 880 to 2281 in SEQ ID No 7), resulting in pTS173.

In the claims:

The following claims are amended:

1. (Amended) A [An improved restorer] DNA comprising a barstar [DNA] coding sequence, which, when expressed in a plant

cell, is capable of improved inhibition of barnase, wherein said barstar coding sequence [DNA] has an AT content of less than 40%.

9. (Amended) The process of claim 8, wherein said promoter is the promoter of the TA29 gene of tobacco, the promoter of CA55 gene of corn or the promoter of the E1, the T72 or the T42 gene of rice.